The Division of the Collar-Cells of Clathrina coriacea (Montagu): A Contribution to the Theory of the Centrosome and Blepharoplast.

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With Plates 25 and 26.

Introductory.

At the present time there is a great deal of confusion in the use of the words "blepharoplast" and "centrosome." Two distinct questions arise with regard to the significance of these bodies; the first is the question of the homology of blepharoplasts and centrosomes; the second is that of the nature of the centrosome, and more particularly whether or not it is to be regarded as equivalent primarily to a nucleus.

With regard to the first of these questions, it is now generally admitted that blepharoplasts and centrosomes are essentially bodies of the same nature, for reasons that will presently be considered at greater length. The difference between a centrosome and a blepharoplast, on this view, is entirely a matter of divergence of function. A centrosome may be briefly characterised, in a general way, as a body which exerts or governs kinetic functions in relation to the division of the nucleus; a blepharoplast may be defined as a centrosome which governs the movements of motile organs, such as flagella, which arise from it and are in direct or indirect connection with it.

With regard to the second of these questions, namely, the nature of the centrosome, two opposite views are current,

which may be summarised as follows: (1) The centrosome is to be regarded as primarily a body of achromatic[1] nature, elaborated and evolved, in all probability, in the nucleus or in connection with it, but not itself equivalent to a nucleus; (2) the centrosome is regarded as the equivalent of a nucleus, and as representing primarily a nucleus which has become modified and specialised both in function and structure. These two theories may be termed conveniently the achromatic and the nuclear theory of the centrosome respectively. According to the second of these views, which has recently been revived and advocated by Hartmann and Prowazek (**6**), every cell is to be regarded as primarily and essentially binucleate; the two nuclei, at first, doubtless, equivalent and similar in all respects, became modified in two directions respectively, the one becoming specialised for trophic, the other for kinetic functions, with corresponding differentiation of structure. In the metazoan cell, according to this theory, the nucleus represents the original trophic nucleus deprived of all kinetic structures, while the centrosome represents the kinetic nucleus deprived of all "vegetative" functions and of its chromatic apparatus. On this interpretation of the centrosome, the minute granules which are the centre of kinetic functions are termed "centrioles," in order to distinguish them from the centrosomes as a whole. In fact, from the point of view of the nuclear theory of the centrosome, the centriole requires to be defined in exactly the same way as the centrosome itself on the achromatic theory.

The confusion produced by these two theories of the centrosome reaches its height in the nomenclature of the different parts of the body of a trypanosome. In these organisms, and in allied genera of flagellates, there are three distincts parts of the nuclear apparatus to be reckoned with. First, there

[1] Meaning by the term "achromatic" something which is not composed of chromatin, not necessarily something which is not coloured by stains. All is not chromatin that stains, even with a so-called nuclear stain. In our opinion a great deal of error and misconception has arisen by identifying as "chromatin" all bodies in the cell that are coloured black, for instance, by the iron-hæmatoxylin method.

is a chromatic body, which may be denoted temporarily by the symbol *N*, situated usually in or near the middle of the cell-body, and in no special connection with the flagellar apparatus. Secondly, there is a second chromatic body, which may be denoted by the symbol *n*, distinctly connected with the flagellum or flagella, when they are present, and apparently kinetic in function. In the genera Trypanosoma, Herpetomonas, Leishmania, and Crithidia, *N* is always much larger than *n*, but in Trypanoplasma the reverse may be the case. Finally, the flagella arise, probably in all cases, from basal granules, which are often very minute and exhibit staining reactions quite different from either *N* or *n*.

According to the nuclear theory of the centrosome advocated by Hartmann and Prowazek, these three parts of the trypanosome body are to be interpreted and named as follows: *N* is the trophic nucleus, while *n* represents the second nucleus of kinetic function, in other words, the centrosome, which, since it controls the activities of the flagellar apparatus, is to be termed a blepharoplast. The basal granule is a mere thickening of the proximal end of the flagellum, of no special significance, or at most representing a centriole. Thus a trypanosome would represent the ideal binucleate cell of Hartmann and Prowazek in a very primitive state.

An interpretation of the trypanosome body, quite different to that of Hartmann and Prowazek, has been advocated by one of us (**12**), which may be briefly stated as follows: *N* is a trophic nucleus, which contains its own centrosome or division-centre in itself; *n* is a distinct kinetic nucleus, a specialisation of the nuclear apparatus for a particular function; it has nothing to do with a true centrosome, though it may, like the trophic nucleus, contain a body of this kind, nor is it to be regarded as a blepharoplast, a body which is represented by the basal granule of the flagellum.[1]

[1] It is not our purpose here to summarise the various views that have been put forward with regard to the morphological interpretation of the trypanosome-body, but only to select two which show in sharp

In consequence of these divergent theories and interpretations, a great confusion in nomenclature has arisen, especially with repard to n, which is always termed the blepharoplast in German works, the centrosome in French works, and in this country is sometimes named the micronucleus, but more usually the kinetonucleus.

In Flagellata other than the trypanosomes and their allies there is usually only one structural element other than the principal nucleus (N) to be reckoned with in the nuclear apparatus, namely, a deeply staining grain or set of grains, from which the flagellum or flagella take origin, and to which the name "blepharoplast," or the synonymous term "diplosome,"[1] is commonly applied. The question at once arises, How is the arrangement seen in a trypanosome to be compared to that of other flagellates, and to which element in the nuclear complex of a trypanosome should the blepharoplast of an ordinary simple flagellate be compared? Does it represent the basal granule (true blepharoplast, on our view) or the kineto-nucleus (n)? In our opinion, the bodies in question are true blepharoplasts, comparable to the basal granules of the flagella of trypanosomes, and the kineto-nucleus or German blepharoplast of the trypanosomes and their allies is a nuclear body peculiar to them, and not found in ordinary flagellates. To this extent, at least, we are in agreement with the idea expressed by Hartmann, who has placed the trypanosomes and forms regarded as

contrast opposed views with regard to the nature of the blepharoplast and the proper application of this word. Thus Laveran and Mesnil in their well-known work on trypanosomes use the term "centrosome" for n; so also Moore and Breinl, who contrast the extra-nuclear centrosome (n) with the intra-nuclear centrosome (karyosome of N).

[1] "The term "diplosome," meaning literally and etymologically a double body, is commonly applied, by an abuse of language, to the single grain from which a flagellum arises. It should, of course, be used only for those cases where twin granules give origin to two or more flagella, that is to say it should not be regarded as synonymous with blepharoplast or basal granule, but as implying a condition in which such bodies are doubled.

allied to them in a separate order of Flagellata termed the Binucleata. (The question as to whether or not the Hæmosporidia should be included in the Binucleata is one which, in the present memoir, we do not wish to raise or discuss.) A trypanosome is, in our opinion, a binucleate organism, possessing a trophic nucleus (N), a kinetic nucleus (n), and a blepharoplast (basal granule).

In order to settle these disputed points, more knowledge is required regarding nuclear and other structures connected with the locomotor apparatus in other organisms, and in the hope of throwing some light on these questions we have studied the division of the collar-cells of a calcareous sponge, of which preserved material was in the possession of one of us. A collar-cell, although occurring as tissue-element of a Metazoan organism, is essentially a flagellate organism, comparable in every way with an individual of the Choano-flagellata. It has recently been pointed out by one of us (**13**) that there are two types of collar-cells in calcareous sponges. In one type, characteristic of the family Clathrinidæ, amongst Ascons, the nucleus lies at the base of the cell, far removed from the origin of the flagellum, which arises from a distinct basal granule or blepharoplast situated at the apex of the cell. In the other type, characteristic of the Leucosoleniidæ amongst Ascons, and of the Heterocœla generally, the flagellum arises directly from the pear-shaped nucleus, which is usually situated in the upper part of the cell, close to the point at which the flagellum emerges from the body of the cell. These two differences in arrangement are also paralleled amongst free-living Flagellates, for instance amongst the two genera of Mastigamœbæ described by Goldschmidt (**4**), and there can be no doubt that the conditions are perfectly comparable in the two cases—that is to say, that when the flagellum arises from a basal granule distinct from the nucleus, the basal granules are homologous structures. As the result of our investigations we have obtained, as will be apparent in the sequel, evidence of a most convincing kind as to the identical nature of centrosomes and

blepharoplasts; but before proceeding to the detailed account of our observations it will be useful to give a brief résumé of previous work on this subject. For this we have relied chiefly on the excellent summaries given by Wilson (**20**) and Erhard (**3**).

The most convincing and abundant evidence of the identical nature of blepharoplasts and centrosomes has come from the study of spermatogenesis in animals and plants. These researches have been summarised by Wilson and Erhard, and it will be sufficient here to refer specially to the memoirs of Henneguy (**7**) on the spermatogenesis of Bombyx mori, etc., and of Belajeff (**1**) on that of Gymnogramme and Marsilia spp. Henneguy found, as we have done, the blepharoplast (in this case a diplosome in the true sense of the word) acting as a centrosome in the mitosis while still preserving its function as a blepharoplast. Similarly Belajeff found that the body which acted as a centrosome in the mitosis became subsequently the blepharoplast.

In the case of tissue-cells other than spermatocytes in Metazoa, the relation of flagella and cilia to bodies of centrosomic nature has been studied by Joseph (**9a**), in whose memoir will be found very full references to the work of others. Joseph's researches have led him to support very definitely the theory of Lenhossek and Henneguy, according to which the basal corpuscles of the cilia arise from the centrosome; and in his conclusions he states (l.c., p. 71): "Viele, vielleicht alle eingeisseligen Zellen sind Centralgeisselzellen, d. h. ihr Geisselfaden steht in Verbindung mit dem Centrosom." Erhard (**3**) has reviewed the whole question in the light of renewed investigations, and comes to the following conclusions: "Das Diplosom in Flimmerzellen als Teilungsorgan wirkt, also ein echtes Centrosom darstellt. . . . Die ausserordentliche Seltenheit von Mitosen in Flimmerzellen darauf schliessen lässt, dass die Diplosomen in allgemeinen eine andre Rolle als die der Teilung auszufüllen haben. . . . Zwischen Basalkörpern und Centrosomen keinerlei Beziehungen bestehen . . . die Basalkörpern an der

Teilung der Metazoenflimmerzellen keinerlei aktiven Anteil nehmen, so kann fur diese Zellen die Henneguy-Lenhossekschẹ Theorie nicht mehr aufrechterhalten werden." Thus while maintaining the centrosomic nature of the diplosome, Erhard denies it for the basal granules of the cilia in ciliated cells.

As regards the basal granules of the flagella in Protozoa, evidence bearing on their nature is scanty to a disappointing degree. The majority of investigators appear to ignore these bodies. Schaudinn (**17**) found in *Paramœba* the "Nebenkörper" acting as a centrosome in the mitosis; the flagella of the swarm-spore appear to arise quite independently of the Nebenkörper, a body which, from Schaudinn's investigations, gives the impression of being rather of the nature of a kinetonucleus than of a centrosome (*pace* Hartmann and Prowazek), and which very probably contains its own centrosome (or centriole), which acts also as the centrosome of the principal nucleus in the mitosis. Prowazek (**15**) points out that the flagellum of Flagellata may arise within the nucleus ("Kern-endogener Ursprung") or outside it; in the latter case the flagellum may terminate in a "diplosome," which again may be quite free from the nucleus (as in the collar-cells studied by us) or may be connected with the nucleus by a "rhizoplast." In the nuclear division of *Entosiphon*, Prowazek (**16**) found a "basalkörperartige Verdickung" at the origin of each flagellum, and from this body a rhizoplast passing back to the nucleus. At the division of the nucleus a "Centronukleolusspindel" is formed. The basal granules do not appear to influence the division of the nucleus in any way; they divide, and two new flagella grow out from each pair.

In his famous investigations on the trypanosome of the little owl, Schaudinn (**18**) gives the following account of the origin of the flagellar apparatus. The nucleus of the oökinete contains a karyosome in which a "central grain" is surrounded by eight chromosomes. By heteropolar division the single nucleus divides into a larger nucleus, the trophic nucleus, and a smaller, the kinetonucleus ("blepharoplast"). The kineto-

nucleus is "a complete nucleus with centrosome and eight chromosomes, not merely a centrosome, karyosome, nucleolus, or a simple ectoplasmic thickening." (The contrast drawn between a nucleus and a centrosome in this sentence is instructive.) The kinetonucleus then divides by another heteropolar mitosis and gives rise to a third nucleus, the smallest of the three; this third nucleus forms a nuclear spindle composed of eight mantle-fibres and a "central spindle" or centrodesmose connecting the two centrosomes situated at the two poles of the spindle. The central spindle becomes the flagellum and the eight mantle-fibres the eight myonemes. By growth and elongation of the flagellum and myonemes, one centrosome is carried out at the tip of the flagellum, while the other remains as its basal granule. From these statements of Schaudinn, it may at least be said without expressing any opinion as to the accuracy of the details in the development described by him that he regarded the basal granule of the flagellum as a centrosome, and that he distinguished clearly between a centrosome and a nucleus, and in particular between the kinetonucleus and the centrosomic body from which the flagellum arises, although he used, in our opinion quite wrongly, the term "blepharoplast" to denote the kinetonucleus, instead of applying it to the basal granule of the flagellum. This mistake, as we consider it, in Schaudinn's terminology is the more remarkable, since he seems to have understood so clearly the true centrosomic nature of the basal granule of the flagellum, and to have realised its existence independent of the kinetic nucleus.

The most important contribution to the question of the blepharoplast in the Protozoa is the memoir of Jahn (8) on the swarm-spores of one of the Mycetozoa, Stemonitis flaccida. He finds that at division the centrosomes at the poles of the nuclear spindle give rise to the daughter-flagella while still actually engaged in their centrosomic functions; a state of things entirely parallel to that which we have found in the collar-cells we have studied.

Hamburger (5) found in Dunaliella the paired flagella

arising from a basal granule which is connected with the nucleus. At division the basal granules divide and each gives off two flagella; though they do not appear to control the division of the nucleus in any way, nevertheless each basal granule is connected with the dividing nucleus by two streaks, giving an appearance very similar to that figured by us on Plate 25, figs. 4 and 5. (Jahn also figures a very similar condition.) Dobell (2), in his investigations on Trichomonas, etc., appears to support a view similar to our own. Lastly, Yamamoto (21), who has studied the locomotor apparatus of various organisms by methods which seem to us unduly violent and severe, describes the flagellum of a trypanosome as arising from a basal granule ("proximal centriole"); his statements, in matters of fact, simply confirm those of Schaudinn.[1]

Observations on the Division of the Collar-cells.[2]

The material on which this work was done consists of a number of specimens of Clathrina coriacea preserved by one of us at Roscoff, and embedded in paraffin at the time.

[1] Yamamoto states that he has obtained preparations of trypanosomes (species not stated) showing myoneme fibrillæ, of which he states I deny the existence. This is a glaring misstatement on his part, seeing that I have described and figured the myonemes of Trypanosoma percæ and T. granulosum in full detail (vide 'Proc. Zool. Soc. Lond.,' 1909, pl. v, figs. 84, 96, 97).—E. A. M.

[2] I greatly regret that in my account of the Sponges in Lankester's 'Treatise on Zoology' (Part II, 1900, p. 56) I gave an entirely erroneous account of the division of the collar-cells of Clathrina coriacea, stating that after division of the nucleus the cell divides transversely to its long axis, and then the basal portion forms a new collar and flagellum. I have re-examined the figures and preparations on which these statements were founded, and see that I was misled by sections passing obliquely through the epithelium, so that the top part of a dividing cell, with the nucleus at the apex, appeared superposed on the base of an ordinary cell, with its nucleus in the usual position. The account given in the present memoir will show clearly the error of my former statements.—E. A. M.

Most of the sponges were preserved in osmic acid followed by picrocarmine, a good method for showing clearly the cytoplasmic structures, especially the collar and flagellum, but not suitable for demonstrating the finer details of the nuclear apparatus. Some of the material, however, had been preserved in Hermann's fluid, and it is on this that we base the results set forth in this memoir. Sections cut from sponges preserved in this way were stained with various stains, more particularly by Heidenhain's iron-hæmatoxylin method, and counter-stained with eosin or Lichtgrün, the latter being found to be of great assistance in making out the details of the collar and flagellum, since these parts are tinged by it.

(1) The Resting Collar-cell.—In Clathrina coriacea, as in all sponges of the family Clathrinidæ, the nucleus lies invariably, in the ordinary "vegetative" or resting condition, at the base of the columnar collar-cell, that is to say, at the end which is furthest from the collar and flagellum. At the apex of the cell, in the centre of the area enclosed by the base of the collar, lies a minute granule—the blepharoplast—from which the flagellum takes origin. These structures, no less than the general form of the collar-cell and its position in the epithelium, of which it forms a part, give a definite orientation to the cell; any direction parallel to an imaginary axis continuing that of the flagellum and passing through the blepharoplast and nucleus may be termed vertical, while any plane at right angles to the vertical axis may be termed horizontal.

The form of the collar-cell and the dimensions of their different parts vary considerably with the condition of the sponge, whether expanded or contracted, and may be different also in different parts of the same sponge. In specimens in which the pores are fully open, and in which all appearances indicate that the collar-cells are in full functional activity, the bodies of the cells are fairly broad, and about 12–13 μ in height by 5–6 μ in breadth; the collar reaches a length of 10–11 μ, and the flagellum some 25–27 μ. When, on the other hand, the pores are closed up and the sponge is partially con-

tracted, the collar-cells become taller and narrower and the collar much shorter. In each cell the basal three fourths of the body is broader and more or less cylindrical in shape; this part of the cell is in contact with the neighbouring cells, and constitutes the main body of the cell. We have not found processes connecting the bodies of the cells with one another. It has been shown by Minchin and Reid (**14**) that when the collar-cells are carefully brushed away and the wall of the sponge is stained with picro-nigrosin, a delicate blue-stained network is visible in surface view, representing a honeycomb-like structure, the spaces in which were originally occupied by the bodies of the collar-cells. Hence in life the bodies of the collar-cells are probably not in actual contact, but are separated by a delicate extension of the gelatinous ground-substance of the body-wall of the sponge. If, as would seem probable on theoretical grounds, the bodies of the collar-cells are connected across this intervening substance by protoplasmic fibrils, such connections have escaped our notice, possibly on account of their being of extreme tenuity and requiring, perhaps, other methods of technique, in order to demonstrate their existence, than those employed by us for the study of the mitosis. It is well known that in other sponges the collar-cells may be connected by protoplasmic processes, as, for instance, in Hexactinellids, where such processes are extremely obvious, forming the so-called membrana reticularis.

The cylindrical basal portion of the cell ends in a distinct rim or flange, and from this level arises a narrower portion, which may be termed the "neck," and which is quite free from any contact with neighbouring cells. The summit of the neck is rounded off, forming a convex lens-like area enclosed by the base of the collar, and giving off centrally the flagellum. The so-called collar has more the form of a cuff or sleeve when fully expanded. It is distinctly thicker and more rigid in its basal portion, becoming very delicate at its distal end, which is usually more or less shrunk and distorted in preparations. The uppermost limit of the collar is

often very difficult to make out. It is best preserved in the osmic-picrocarmine preparations; after Hermann's fluid it appears collapsed and shrunk or frayed out. A short way above its origin the collar usually shows a distinct thickening, visible as a horizontal hoop-like structure, especially when the collar is a little contracted; when it is expanded to its fullest extent the hoop is difficult to make out as a horizontal line, but its presence is marked by the fact that all the part of the collar below it stands out stiff and firm, and is not creased and folded like the part above. It is evident from the appearances seen both in the resting and the dividing cell that the collar for about 2 μ from its origin is thickened and strengthened as compared with its distal portion.

The nucleus of the collar-cell is about 5 μ in diameter and more or less spherical in form, sometimes slightly flattened in the vertical direction. The most conspicuous element in its structural composition is a large grain, which stains deeply with iron-hæmatoxylin, and appears to be of the nature of a karyosome. This structure is always present, and sometimes double (figs. 1 *c*, 3, 7). The karysome is sometimes lodged in a clear space (fig. 36, *d*, *e*, *f*); its position in the nucleus varies. The remainder of the nuclear contents appear granular, but in thin sections of the nucleus a fine network can be made out (figs, 36, *e*, *f*, *g*), in the nodes of which the granules of chromatin are lodged. These granules vary very much in different nuclei in the same preparation, being sometimes so fine as to be scarcely visible individually, while in other nuclei they are coarse and irregular in size and shape (figs. 30 and 36, *a*, *b*, *c*). All transitions can be found between the finely and the coarsely granular condition, but the two extremes form two well-marked types, which may be characterised as the light and the dark type respectively. It is worthy of note that nuclei of cells about to divide are always of the light type, as will be pointed out in the next section.

The above description of the nuclei refers to preparations stained with iron-hæmatoxylin. In material preserved and

stained by the osmic-picrocarmine method the nuclear structure is not shown at all as a rule, but the nucleus simply stains evenly pink. Sometimes the karyosome can just be made out, sometimes not. A peculiar feature of the preparations is that the red stain often does not extend up to the nuclear membrane; the stained portion forms a mass lying in the centre of the nucleus, and between this stained mass and the nuclear membrane a clear space remains, which can often be seen to be traversed by delicate radiating lines, as if fine filaments started from the membrane to support the central stained mass. Comparison with nuclei stained with iron-hæmatoxylin shows in many of the latter a distinct alveolar border to the linin-framework; sometimes the alveolar border is relatively very broad (fig. 36 *e*), and shows the radiating partitions of the alveoli very distinctly. It would appear as if the action of the osmic-picrocarmine method was to cause a shrinkage within the alveolar border, with the result that this inner portion of the nuclear framework contracts and appears as a homogeneous mass, which contains all the chromatin and stains deeply, leaving the alveolar border unstained. It should be noted that by no means all the nuclei of the collar-cells show the clear border within the membrane; many of them stain evenly up to the membrane, and this is always so in those cells which are about to divide.

The blepharoplast and flagellum stain black with iron-hæmatoxylin, but by the osmic-picrocarmine method they are not stained.

The cytoplasm of the collar-cells is finely granular and usually very vacuolated. The neck is free from vacuoles as a rule, but in many cases a round vacuole-like structure, which differs in appearance from the other vacuoles, can be seen in the neck region. The ordinary vacuoles in the body of the cell are clear and appear as empty spaces, doubtless representing drops of fluid in the living condition, but in the direct line between the nucleus and blepharoplast there is generally to be seen a vacuole, which has finely granular

contents and sometimes a minute central granule (fig. 30, cell on the extreme right). This body is sometimes nearer the blepharoplast, sometimes nearer the nucleus, but usually it lies at a level midway between the neck and the main body of the cell or in the neck itself; its significance is doubtful.

In addition to the vacuoles, the cytoplasm almost always contains one or more coarse refringent granules of irregular, angular form and yellowish-brown colour. They are lodged in any part of the cell and are often present in the vicinity of the blepharoplast. They probably represent excretion-grains. After the iron-hæmatoxylin stain they become darker, but still retain their characteristic yellowish-brown tint, and can be easily distinguished from chromatin grains. No other enclosures, as a rule, are to be found in the collar-cells, but occasionally they contain large rounded bodies (figs. 31–35 and 50, 51), which stain deeply with iron-hæmatoxylin and appear to be of the nature of organisms, though whether they represent parasites or food ingested by the cells is difficult to say. In some parts of the sponge they are found more commonly than in others, and in one case (fig. 34) no nucleus could be made out in the cell; it may, however, have been cut off in the section.

(2) Preparations for Division.—Before the nucleus begins to show any of the changes in its minute structure which initiate mitosis certain events take place in the cell, namely, the migration of the nucleus bodily from the base to the summit of the cell, the disappearance of the flagellum, and the division of the blepharoplast. As a general rule these three events take place in the order named, but not invariably, so that a number of different combinations arise in different cases.

The migration of the nucleus is always the first sign that a collar-cell is about to divide, and this peculiarity is a great aid to the study of the division, since in a section of the sponge which shows the collar-cells cut vertically those that are dividing or preparing to divide arrest the attention at once, even with a comparatively low power of the microscope,

owing to the fact that the nucleus is no longer in its usual position at the base of the cell, but has either migrated to the apex or has been preserved in the act of doing so, and is found in some position between the base and the apex (figs. 1–5, etc.). Such cells are also characterised by being much broader and stouter than the ordinary resting cells, but they do not increase in height to an appreciable extent.

By this process of migration the nucleus comes to lie immediately under the blepharoplast, and at this stage a curious appearance has been observed in two instances (figs. 4 and 5); the nucleus is seen to be flattened on the side nearest to the blepharoplast, and from the blepharoplast itself two streaks appear to radiate to the two ends of the flattened side of the nucleus. Careful examination of each of these preparations gives the impression that these two streaks are in reality the optical section of a cone-shaped mass of protoplasmic substance, the base of which rests on the flattened side of the nucleus, and which is, perhaps, the cause of the flattening. A comparison with the resting cell suggests that this conical mass is derived from the peculiar vacuole with granular contents, which was described in the last section as situated in the direct line between nucleus and blepharoplast, and that by the upward migration of the nucleus the vacuole in question is pushed up until it is caught, so to speak, between nucleus and blepharoplast, when, coming under the influence of the forces of attraction or repulsion exerted by the blepharoplast, it assumes the conical form seen. If this is a correct interpretation of the phenomena, the vacuole should, perhaps, be regarded as an archoplasmic vesicle, such as has been described in other cases, and which supplies some part of the material of the achromatic spindle in the mitosis. In fig. 4 it is seen that the flagellum is still present, though short, while in fig. 5 the flagellum has entirely disappeared and the blepharoplast has divided.

The disappearance of the flagellum and the division of the blepharoplast are two events which take place independently so far as their relative sequence in time is concerned, that is

to say, the flagellum may disappear completely before the blepharoplast divides or may persist until after this has taken place. In either case the two daughter-blepharoplasts migrate inwards and place themselves on opposite sides of the nucleus in order to become, as will be seen, the two centrosomes in the mitosis. If the flagellum persists during this process of events it remains attached to one of the two blepharoplasts (figs. 6 and 10), and becomes drawn into the body of the cell, as seen in figs. 7–9; in each of these three specimens the flagellum, though greatly shortened, is still persistent, and can be seen passing into the body of the collar-cell and terminating in one of the two blepharoplasts, while the other blepharoplast can be seen on the other side of the nucleus quite independent of the flagellum. On the other hand, figs. 5, 11, and 12 show the two blepharoplasts very close together at the apex of the cell and apparently very recently separated from one another, with no trace of a flagellum.

The exact method in which the flagellum disappears is difficult to determine simply by comparison of different stages in sections; it could only be made out satisfactorily by watching the process in the living cell. In collar-cells in which the upward migration of the nucleus is taking place, the flagellum almost always appears much shorter than in the surrounding cells, an appearance too constant in occurrence to be explained simply as due to artificial curtailment of the flagellum in the process of section-cutting, especially when the collar is intact and the flagellum does not project beyond it (figs. 9 and 10). But a remarkable feature of this stage is the frequent occurrence of a protoplasmic projection, like a small pseudopodium, from the apex of the cell round the base of the flagellum (figs. 7, 9, 39, 40); this process persists for a time after the flagellum has completely disappeared (figs. 14, 41). The appearances suggest that the cell throws out a pseudopodial process, by the help of which the flagellum is retracted and absorbed at its base;

in all cases the protoplasmic process in question is very short in proportion to the length of the original flagellum.

The division of the blepharoplast takes place with formation of a distinct centrodesmose connecting the two daughter-blepharoplasts (figs. 6, 7, 13).

During these changes the collar remains practically unaltered, except that it begins to show more or less clearly the appearance of shrinkage and degeneration characteristic of the succeeding stages of the division.

(3) The Mitosis.—The general course of the mitosis in the collar-cell is similar to that known to occur in the cells of other Metazoa generally, and described for sponges by Maas (**10**, **11**) and Jörgensen (**9**). It is unnecessary, therefore, to do more than describe its most characteristic features.

As already stated in a previous section, the nucleus of a collar-cell about to divide, but before any changes preparatory to division have begun in the chromatin contents, is of a pale type—that is to say, the granules of chromatin distributed over the general framework are very fine and scattered evenly, so as to give the nucleus an almost homogeneous appearance relieved only by the karyosome, stained a deep black, after iron-hæmatoxylin, in contrast with the pale grey tint of the remainder of the nucleus (figs. 1, 10, 11). The nucleus at this stage is also distinctly larger than the average nucleus of a resting cell.

The first changes to be observed in the chromatin contents of the nucleus are that they stain darker and become more blotchy and uneven in appearance, apparently as the result of the minute granules of chromatin being clumped together to form coarse grains or masses. Figs. 6 and 7 show early stages in this process; the masses of chromatin still stain faintly, appearing to be loose in texture and ill-defined in outline, and the karyosome stands out sharply. In later stages (figs. 8, 12) the chromatin masses become more definite in outline and somewhat smaller, and the deep stain they take gives the impression that they are more closely knit and of denser texture; the karyosome, however, is still distinct.

Finally, the chromatin masses become very definite and stain very deeply, and no distinct karyosome can be made out; this body seems to break up and to contribute by doing so to the general store of chromatin. At first the chromatin masses, or chromosomes, as they may now be termed, appear to be connected together by delicate filamentous junctions (fig. 9); this stage corresponds apparently to the spireme stage. Next, the connections between the chromosome disappear, and they are seen lying separately from one another as irregular rounded masses, showing more or less distinctly indications of division, each into two (fig. 15). In spite of much searching we have not been able to find any stages other than those described, and, in particular, nothing more nearly resembling an ordinary spireme stage than the specimen shown in fig. 9.

These changes in the interior of the nucleus also go on quite independently of the changes in the flagellum and blepharoplast described in the previous section. Thus the flagellum may have vanished, and the two daughter-blepharoplasts may have taken up their definitive position when the nuclear contents are at the beginning of their changes (fig. 14); or the nucleus may be comparatively far advanced when the blepharoplast has only just divided (fig. 12), or before the flagellum is absorbed (figs. 8, 9). Finally, however, a stage is reached when the nucleus has resolved itself into a mass of separate chromosomes, and the two blepharoplasts, or, as they may now be termed, the centrosomes, are placed on opposite sides of it, indicating the two poles of the future nuclear spindle (fig. 15); when this stage is reached the nuclear membrane is absorbed and cannot be discerned.

The formation of the nuclear spindle is seen in the two stages drawn in figs. 16 and 17. After the absorption of the nuclear membrane the chromosomes arrange themselves to form an equatorial plate, to which delicate rays can be seen to pass from the centrosomes, forming the characteristic achromatic spindle. The two centrosomes appear to be pushed further apart by the formation of the spindle, so that they

come to lie at the extreme surface of the cell. The spindle is lodged in that portion of the cell which we have termed the neck in a previous section, and the centrosomes are situated about midway between the origin of the still persistent collar and the flange. The chromosomes appear massed together, and are difficult to distinguish individually when the equatorial plate is seen in side view (figs. 17, 18), but can be seen better in cells cut parallel to the plane of the equatorial plate (fig. 22). The number of chromosomes appears to be about sixteen.

At this period, while the equatorial plate is still simple and undivided, an important event takes place. From the centrosomes at the two poles of the spindle the two daughter-flagella grow out, appearing as two minute hair-like projections from the surface of the cell (figs. 18–21). This stage is a very common one, and it is, in fact, rare to find a collar-cell with a mitotic spindle without the two daughter-flagella projecting from the two centrosomes; this indicates that the first formation of the flagella must be an extremely rapid one. Sometimes only one daughter-flagellum is to be seen, but in such cases the cell is usually slightly oblique, and the missing flagellum has probably been cut off by the knife in cutting the section. The two new flagella are formed entirely outside the original collar, which is still persistent. The condition of the collar is best studied in osmic-picrocarmine preparations (figs. 42–45), in which it is seen that the formation of the nuclear spindle causes the cell to become much broader, with the result that the base of the collar is greatly stretched. The thicker portion of the collar, below the hoop, retains its form more or less, but the portion above the hoop tends to collapse and fall together.

From the stage with the single equatorial plate the diaster-stage arises in the usual way (figs. 23, 24). It is remarkable that we have succeeded in finding but few specimens of the diaster-stage, and, unfortunately, most of those have been cut obliquely or horizontally, and hence do not show well the relation of this stage to the cell as a whole. Figs. 23 and 24

show the two best diaster-stages we have found. Fig. 23 shows the spindle well, but the cell is cut almost horizontally, and the collar and one daughter-flagellum are sliced off; in fig. 24 the cell is cut more vertically, and shows the collar, but the plane of the spindle lies obliquely, and only one centrosome and daughter-flagellum can be made out. The scarcity of the diaster-stage indicates that it is passed over very rapidly, and this conclusion receives further support from the fact that in the subsequent stages, when the daughter-nuclei are being reconstituted, the daughter-flagella are scarcely longer than they were in the stage with the undivided equatorial plate.

After the diaster-stage, and with the reconstitution of the daughter-nuclei, the cell-body begins to divide (figs. 25–28A). Between the two daughter-nuclei there are seen for a time streaks, the remains of the achromatic spindle, stretching across from one to the other (figs. 25–27); these streaks persist until the division of the cell-body is far advanced. The details of the reconstitution of the nuclei are difficult to make out clearly; the chromosomes appear to fuse together into a compact mass in which their individuality is masked, if not lost. The division of the cell is effected by means of a constriction in the vertical plane, producing a cleavage which is much more marked at the upper than at the lower end of the cell. The cleavage goes right through the old collar, and leads to its destruction and disappearance; it appears to break down into a granular mass which disintegrates and vanishes.

When division of the cell-body is complete the new collars of the daughter-cells grow out round the short but growing flagella. At their first origin the new flagella projected in an oblique direction from the dividing cell, as figs. 18–28 show clearly; they took origin from that portion of the surface of the parent-cell which lies between the flange and the base of the collar. When the division is nearly complete (figs. 28A and 47), the point of origin of the flagella becomes slightly shifted so as to be placed at the uppermost level of

the cell, with the result that the young flagella come to point vertically upwards. After complete division the form of the two daughter-collar-cells undergoes a change, becoming elongated in the vertical direction, so that the cell as a whole acquires a slender columnar form, with a shallow collar surrounding the short flagellum at the upper end (figs. 29, 30, 48). A curious feature of these stages, both those in which cleavage of the cell is taking place (figs. 25–28A) and those in which division is recently completed (figs. 29, 30, 48), is that they are found in the sections at a higher level than the rest of the epithelium, as shown in figs. 30 and 48; the bases of the young cell are on a level with the flanges of the ordinary resting collar-cells. This peculiarity is very marked when the recently divided cells have assumed the columnar form; they project so much above the general level of the collared epithelium that they become very conspicuous objects in the sections of the sponge, and are consequently very easy to find. Later they appear to push their way down amongst the other epithelial cells, and so find their normal level (fig. 49).

The nuclei of the young collar-cells, at first compact masses, soon become looser in texture; the karyosome reappears and nucleus acquires the structure of the ordinary resting nuclei, from which it differs only in its smaller size. In osmic-picrocarmine preparations the young nuclei show the marginal clear zone very distinctly (figs. 48, 49). Immediately after division the nucleus is at the apex of the collar-cell (figs. 29, 30, 48), but it now begins to migrate towards the base of the cell (fig. 49), and so resumes the position characteristic of the resting cell. The collar and flagellum grow to their full length, and the latter arises from a basal granule or blepharoplast which, as is clear from the development that has been described and depicted, is one of the two centrosomes of the nuclear spindle in the mitosis, derived from the division of the resting cell.

Summary and Conclusions.

The course of events that take place in the division of the collar-cells may be summarised briefly as follows, omitting the details of the mitosis, since they present no special peculiarities.

The nucleus of the collar-cell migrates from the base to the apex of the cell, and so comes to lie immediately under the blepharoplast. The flagellum then disappears and the blepharoplast divides. The two daughter-blepharoplasts travel to opposite sides of the nucleus and take on the function of centrosomes. The nucleus breaks up into chromosomes, its membrane disappears, and a mitotic spindle is formed in the ordinary way, with the two centrosomes at its poles. The two new flagella then at once begin to grow out from the two centrosomes, outside the original collar, before the equatorial plate is divided. The mitosis is completed, and as the cell-body divides the original collar breaks down and disappears. The centrosomes become the blepharoplasts of the two daughter-cells, the flagella continue to grow out from them, the new collars grow up round the new flagella, the reconstituted daughter-nuclei migrate back again to the bases of the cells, and the two daughter-cells resume the structure and appearance of the ordinary resting collar-cells. Thus it is seen that the blepharoplast-centrosome is a permanent cell-organ, which multiplies with the cell; but that the collar and flagellum are formed afresh at each cell-division, quite independently of the collar and flagellum of the parent cell.

In this process of division the feature to which we wish to draw special attention is the fact that the bodies which have the function of blepharoplasts in the resting-cell have that of centrosomes in the dividing cell. In fact, it is seen that during a certain stage in the division, the stage, namely, of the nuclear spindle, when the daughter-flagella are growing out from the centrosomes at the poles of the spindle, one and

the same body functions at one and the same time as a blepharoplast and a centrosome, thus furnishing a decisive proof of the identical nature of these bodies, at least in the class of cells that we have been studying.

We are therefore in entire agreement with those authors who regard blepharoplasts as bodies of centrosomic nature. It is very obvious in the case which we have studied that the terms "blepharoplast" and "centrosome" denote merely two different functional activities of the same body. It may well be that in other cases division of labour may lead to structural differentiation, and that two distinct and independent classes of bodies occur, centrosomes controlling nuclear division and blepharoplasts giving rise to locomotor cell-organs. But in all cases alike we regard centrosomes and blepharoplasts as organs similar in nature and identical in phyletic origin.

It only remains to discuss how far the results we have obtained throw light on the state of things in other cases, and more particularly with regard to the vexed question of the true blepharoplast in trypanosomes, that is to say, whether the name "blepharoplast" should be given to the kinetonucleus, or to the basal granule of the flagellum in these organisms. With regard to this point, it may be stated at once that there is nothing whatever in the structure or behaviour of the centrosome-blepharoplast of the collar-cells to justify a comparison between it and the kinetonucleus of a trypanosome, or, indeed, a nucleus of any kind. We are fully in agreement with those who, following Schaudinn, regard the kinetonucleus of trypanosomes as a body of the nature of a nucleus, and it is precisely on this ground that we regard it as a body of a different nature from a true blepharoplast, such as that which is seen in the collar-cells, and which cannot, in our opinion, be identified as a nucleus by any stretch of the imagination. On the other hand, the body, which in a trypanosome corresponds in every way to the true blepharoplast, is the basal granule or centriole of the flagellum.

Our position, therefore, with regard to the nuclear apparatus

of a trypanosome is that the basal granule of the flagellum represents the true blepharoplast, a body of the nature of a centrosome, and that the kinetonucleus or German blepharoplast is an accessory nucleus which is not represented in the economy of a collar-cell or in flagellated organisms generally, but which is a special feature of the genus Trypanosoma and its allies, especially the genera Trypanoplasma, Herpetomonas, Leishmania, and Crithidia, a nucleus which doubtless possesses its own centrosome or centriole. With regard to the function of the kinetonucleus, its close association with the blepharoplast and the flagellar apparatus has generally been held sufficient to justify the assumption that it possesses a kinetic function, that is to say, that it is a nucleus specially concerned with the regulation of the function of locomotion. We require, however, more knowledge with regard to the relations of the kinetonucleus to the life-cycle as a whole, and more particularly to the phenomena of sex and sexual conjugation in these flagellates before this point can be decided. We may refer in this connection to the interesting experiments of Werbitzki (**19**), who was able to obtain trypanosomes without a kinetonucleus (termed by him "blepharoblast"), and found that such individuals showed no difference, as regards their movements, from the trypanosomes of normal structure. This result seems to us to indicate that the flagellar apparatus of a trypanosome is not so dependent on the kinetonucleus as is generally supposed, and also to be strongly in favour of our view that the basal granule of the flagellum, and not the kinetonucleus, represents the true blepharoplast. Werbitzki seems, in fact, to have reduced his trypanosomes artificially to the more primitive condition found in other flagellates and also in collar-cells, a condition in which the organism possesses a nucleus and a true blepharoplast, but no kinetonucleus.

It may be objected to our conclusions that they are based only on analogy, and that a collar-cell is too far removed from a trypanosome in phylogeny and affinities to permit of

conclusions being drawn with regard to the homologies of the flagellar apparatus of trypanosomes. It is, of course, possible that the conclusions drawn from the one do not strictly apply to the other, and it is certainly very desirable that these points should be studied in flagellates generally, and in forms allied to trypanosomes particularly, more than has been done at present. On the other hand a collar-cell, although it forms part of the epithelium of a sponge, is as much a flagellate organism in all points of structure and function as any free-living flagellate; and the study of cytology tends rather to demonstrate the essentially uniform nature of permanent cell-structures throughout the whole range of living organisms, whether animal or vegetable.

Lister Institute, Chelsea, S.W.
April 26th, 1910.

References.

1. Belajeff, W.—"Ueber die Centrosome in den spermatogenen Zellen," 'Ber. Deutsch. botan. Ges.,' xvii, 1899, pp. 199–205, pl. xv.

2. Dobell, C. C.—"Researches on the Intestinal Protozoa of Frogs and Toads," 'Quart. Journ. Micr. Sci.,' 53, 1909, pp. 201–277, pls. 2–5, 1 text-fig.

3. Erhard, H.—"Studien über Flimmerzellen," 'Arch. f. Zellforschung,' iv, 1910, pp. 309–442, pls. xxii, xxiii, 16 text-figs.

4. Goldtschmidt, R.—"Lebensgeschichte der Mastigamöben Mastigella vitrea n. sp. und Mastigina setosa n. sp.," 'Arch. Protistenk.,' suppl. i, 1907, pp. 83–168, pls. v–ix, text-figs. A–U.

5. Hamburger, C.—"Zur Kenntnis der Dunaliella salina, etc.," 'Arch. Protistenk.,' vi, 1905, pp. 111–130, pl. vi, 7 text-figs.

6. Hartmann, M., and Prowazek, S. v.—"Blepharoplast, Caryosom, und Centrosom," 'Arch. Protistenk.,' x, 1907, pp. 306–335, 8 text-figs.

7. Henneguy, L. F.—"Sur les rapports des cils vibratiles avec les centrosomes," 'Arch. d'Anat. Micr.,' i, 1898.

8. Jahn, E.—"Myxomycetenstudien 3," 'Ber. Deutsch. botan. Ges.,' xxii, 1904, pp. 84–92, pl. vi.

9. Jörgensen, M.—"Beiträge zur Kenntnis der Eibildung, Reifung, Befruchtung und Furchung bei Schwämmen (Syconen)," 'Arch. f. Zellforschung,' iv, 1910, pp. 163–242, pls. xi–xv, 1 text-fig.

9A. Joseph, H.—"Beiträge zur Flimmerzellen und Centrosomenfrage," 'Arb. Zool. Inst. Wien,' xiv, 1903, pp. 1-80, 3 pls., 3 text-figs.

10. Maas, O.—"Ueber Reifung und Befruchtung bei Spongien," 'Anat. Anzeiger,' xvi, 1899, pp. 290–298, 12 text-figs.

11. ——— "Die Weiterentwicklung der Syconen nach der Metamorphose," 'Zeitschr. wiss. Zool.,' lxvii, 1900, pp. 215–240, pls. ix–xii.

12. Minchin, E. A.—"Investigations on the Development of Trypanosomes in Tsetse-flies and other Diptera," 'Quart. Journ. Micr. Sci.,' 52, 1908, pp. 159–260, pls. 8–13, 2 text-figs.

13. ——— "The Relation of the Flagellum to the Collar-cells of Calcareous Sponges," 'Zool. Anzeiger,' xxxv, 1909, pp. 227–231, 6 text-figs.

14. ——— and Reid, D. J.—"Observations on the Minute Structure of the Spicules of Calcareous Sponges," 'Proc. Zool. Soc., London,' 1908, pp. 661–676, pls. xxxiv–xxxvii.

15. Prowazek, S. v.—"Flagellatenstudien," 'Arch. Protistenk.,' ii, 1903, pp. 195–212, pls. v, vi.

16. ——— "Die Kernteilung des Entosiphon," 'Arch. Protistenk., ii, 1903, pp. 325–328, 12 text-figs.

17. Schaudinn, F.—"Über den Zeugungskreis von Paramœba eilhardi," 'Sitz. ber. Akad. Wiss. Berlin,' 1896, pp. 31–41, 12 text-figs.

18. ——— "Generation- sund Wirtswechsel bei Trypanosoma und Spirochæte," 'Arb. k. Gesundheitsamt., Berlin,' xx, 1904, pp. 387–439, 20 text-figs.

19. Werbitzki, F. W.—"Ueber blepharoblastlose Trypanosomen," 'CB. Bakt. Parasitenkunde,' 1 Abth. Orig. liii, 1910, pp. 303–315, pls. i, ii, 6 text-figs.

20. Wilson, E. B.—'The Cell in Development and Inheritance,' 2nd edition, 1904.

21. Yamamoto, J.—"Ueber den Lokomotionsapparat der Protistenzellen," 'CB. Bakt. Parasitenkunde,' 1 Abth. Orig. liii, 1909, pp. 38–42, 1 pl., 1 text-fig.

EXPLANATION OF PLATES 25 AND 26.

Illustrating Miss Muriel Robertson and Mr. E. A. Minchin's paper on "The Division of the Collar-cells of Clathrina coriacea (Montagu): A Contribution to the Theory of the Centrosome and Blepharoplast."

[All the figures are drawn from sections of material fixed with Hermann's fluid and stained by Heidenhain's iron-hæmatoxylin method; the outlines were traced with the camera lucida at a magnification of 2000 linear, with the exception of figs. 36 and 37, which are magnified 3000 linear.]

PLATE 25.

Fig. 1.—Six collar-cells in their natural arrangement; five of them are in the resting state; the sixth (*d*) shows the nucleus in its migration towards the blepharoplast, and has a very short flagellum.

Fig. 2.—Early stage in the upward migration of the nucleus; the blepharoplast in the act of division, with shortened flagellum.

Fig. 3.—Similar stage to the last, the blepharoplast distinctly divided, the nucleus with two karyosomes.

Figs. 4 and 5.—Stages showing the nucleus in close proximity to the blepharoplast and distinctly flattened on the side nearest the blepharoplast, from which two streaks are seen to come down to the two ends of the flattened border of the nucleus; these two streaks appear to be the optical section of a cone-shaped figure. In fig. 4 the flagellum is seen to be still present, but shortened; in fig. 5 no flagellum is seen and the blepharoplast is divided.

Fig. 6.—Stage showing the divided blepharoplasts connected by a centrodesmose; the flagellum is still present and of fair length.

Figs. 7, 8, and 9.—Stages showing complete division of the blepharoplast with persistent flagellum in each case; the two daughter-blepharoplasts in each dividing cell have travelled inwards and placed themselves at opposite sides of the nucleus, drawing in with them the root of the flagellum, and the free portion of the flagellum has its base surrounded by an upgrowth from the apex of the cell. In fig. 7 the adjacent resting cell is drawn for comparison; in the dividing cell a centrodesmose is seen between the two blepharoplasts, and there are also indications of a streak running down from one of the blepharoplasts to a granule in the body of the cell, but this streak appears to be due merely to the arrangement of vacuoles in the cytoplasm, and not of the nature of a

centredosmose. These three figures also show three different conditions of the nucleus preparatory to mitosis. In fig. 7 the karyosome is very distinct, while the remainder of the chromatin is pale, but beginning to aggregate into larger masses. In fig. 8 the karyosome is also distinct, but the rest of the chromatin is darker and the coarse granulation is more distinct. Fig. 9 shows the stage which appears to correspond to the spireme-stage; the chromatin is in darkly staining masses (chromosomes), connected by fainter lines, and no karyosome can be made out. All three cells are from the same slide.

Fig. 10.—Blepharoplast divided, remnant of flagellum still present; nucleus not showing any preparation for mitosis. Cell cut somewhat obliquely.

Fig. 11.—Blepharoplast divided, flagellum entirely absent; nucleus as in last.

Fig. 12.—Blepharoplast and flagellum as in last; nucleus showing beginning chromosome-formation, but karyosome still distinct.

Fig. 13.—Cell cut obliquely, showing two blepharoplasts connected by a centrodesmose.

Fig. 14.—Cell showing the flagellum completely withdrawn, and represented only by a little upgrowth from the body of the cell; the two blepharoplasts (centrosomes) have placed themselves on opposite sides of the nucleus, which is still in a very early stage of preparation for mitosis, with distinct karyosome and pale chromatin.

Fig. 15.—Similar stage, but with the chromatin of the nucleus completely broken up into chromosomes. No karyosome is to be made out. One centrosome is seen on the right at the side of the nucleus, the other on the left, rather low down and almost under the nucleus.

Figs. 16, 17.—Stages showing the formation of the nuclear spindle. In fig. 16 the chromosomes are still irregular in arrangement, while in fig. 17 they are arranged to form a definite equatorial plate. No flagella have as yet grown out from the centrosomes.

Figs. 18–21.—Stages with the nuclear spindle and with daughter-flagella growing out from the centrosomes (blepharoplasts). In fig. 18 the spindle lies slightly obliquely, and only one daughter-flagellum is seen. In fig. 21 the cell is cut obliquely.

Fig. 22.—Nuclear spindle cut in the plane of the equatorial plate, which is seen from one of its flat surfaces.

Figs. 23, 24.—Diaster-stages. Fig. 23 shows a cell cut obliquely, and only one of the daughter-flagella is seen. In fig. 24 the nuclear spindle lies obliquely, and only the left-hand centrosome and daughter-flagellum can be seen.

Fig. 25.—Late diaster-stage, with beginning reconstitution of the daughter-nuclei. Slightly oblique; only one daughter-flagellum to be seen.

Figs. 26–28, 28A.—Stages in the division of the cell-body, with reconstitution of the daughter-nuclei. In all figures, except 28A, the remains of the original collar can be seen clearly. In figs. 26 and 27 the remains of the achromatic spindle can be seen between the two daughter-nuclei. In fig. 28A the division is practically complete.

Figs. 29, 30.—Pairs of young, recently divided collar-cells. In fig. 30 some of the adjacent cells are drawn to show the way in which the cells at this stage are raised up above the surrounding cells.

Figs. 31–35.—Collar-cells showing enclosures of various kinds, some of them perhaps of parasitic nature. In the cell shown in fig. 34 the nucleus seems to have disappeared, but may have been cut off.

Fig. 36.—Nuclei of resting collar-cells, magnified 3000 linear. *a, b, c*, dark nuclei; *d*, a light nucleus; *e, f, g*, thin sections of nuclei showing the reticular structure; in *g* the karyosome does not come into the section.

Fig. 37.—Transverse sections of collar-cells in the region of the collar. *a* passes through the base of the collar, and *b* just above this level; both show the blepharoplast centrally. In *c* the collar is cut transversely with the flagellum in the centre.

PLATE 26.

[All the figures are drawn from sections of material fixed with osmic acid and stained with picro-carmine; magnification throughout 2000 linear.]

Fig. 38.—Two collar-cells, one of the normal resting type (on the left), the other with the nucleus migrating towards the apex of the cell preparatory to division.

Figs. 39, 40.—Collar-cells showing the nucleus at the apex of the cell, and the flagellum in process of retraction by means of a pseudopodium-like process from the cell.

Fig. 41.—On the left a normal resting cell; on the right a cell with the nucleus at the apex and the flagellum completely retracted, but represented by the still persistent pseudopodium-like process seen in the two preceding figures.

Figs. 42, 43.—Stages with the daughter-flagella growing out from the poles of the nuclear spindle, and with the collar beginning to collapse. The achromatic elements, namely, spindle and centrosomes, are not stained and are not visible in the preparation, but the equatorial plate

is seen. In fig. 43 the collar contains a foreign body, as in the right-hand cell in fig. 48.

Figs. 44–46.—Diaster-stages with daughter-flagella. In fig. 44 a resting cell is drawn for comparison; in fig. 46 the cell is cut obliquely and does not show the collar.

Figs. 46A, 47.—Stages in the division of the cell-body. In fig. 46A the collar is still seen; in fig. 47 it has disappeared.

Fig. 48.—Two young, recently divided collar-cells, drawn with three ordinary resting collar-cells to show the manner in which the young cells project above the level of the epithelium. The collar-cell on the extreme right shows a foreign body lodged in the lumen of the collar.

Fig. 49.—Four collar-cells, of which the two middle ones are evidently a pair of sister-cells, the product of recent division, showing the nuclei in the act of migrating down to the base of the cell.

Figs. 50, 51.—Two collar-cells showing bodies (parasites?) in the cytoplasm.